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RETINAL DISORDERS

Intravitreal bevacizumab in active progressive proliferative diabetic retinopathy

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Abstract
Background Vitreous concentration of vascular endothelial growth factor (VEGF) rises significantly during proliferative diabetic retinopathy (PDR). Bevacizumab (Avastin) is a humanized monoclonal antibody to VEGF. Intravitreal administration of bevacizumab (IVB) has recently been shown to be effective in some ocular neovascularizations, including PDR. In this study we evaluate the efficacy of IVB in eyes with active, progressive PDR.
Methods In an interventional prospective case series, eyes with active, progressive PDR underwent one to three IVB injections (1.25 mg) at intervals of either 6 or 12 weeks. Complete ophthalmic examinations and color fundus photography were performed at baseline and 1, 6, 12, and 20 weeks after the first injection. Fluorescein angiography (FA) was performed before injection and 20 weeks after. The primary outcome measures were clearing of vitreous hemorrhage (VH) and regression of active fibrovascular tissue (FVT). The secondary outcomes were any change in best-corrected visual acuity (BCVA) and any incidence of adverse events.
Results Thirty eight eyes of 38 patients with a mean age of 54.7±10.1 years were included in the study. VH resolved significantly after 1 week (P=0.014), 12 weeks (P=0.0001), and 20 weeks (P=0.002). The vascular component of FVT regressed, though the FVT area did not change. Mean BCVA improved significantly compared to baseline at all follow-up examinations. Two cases showing moderate fibrous proliferation developed traction retinal detachment (TRD).
Conclusions IVB has significant therapeutic effect on eyes with active, progressive PDR: the treatment causes a significant amount of VH resolution and neovessel regression. At the same time, this procedure may increase the risk of TRD in eyes with fibrous proliferation.

Keywords Active progressive PDR · Fibrovascular tissue · Intravitreal bevacizumab · Vitreous hemorrhage

The authors have no proprietary interest in this study.

The authors have full control of all primary data, and they agree to allow Graefes Archive for Clinical and Experimental Ophthalmology to review their data upon request.

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Introduction

Panretinal photocoagulation (PRP) is the standard treatment for proliferative diabetic retinopathy (PDR). However, laser treatment of this progressive vasoproliferative disorder fails to cause disease regression in 40% of cases [1]. Early vitrectomy to provide media clarity and remove the fibrovascular tissue (FVT) has been suggested for these difficult cases [2, 3]. Despite laser therapy and vitreous surgery, severe visual loss may occur in cases of progressive diabetic retinopathy. Thus, researchers have focused on pharmacologic treatment for such cases [4]. Octreotide, a somatostatin analog with growth hormone inhibitory and antiproliferative effects, has been proposed as a way to inhibit retinal neovascularization [5, 6].

The key role of vascular endothelial growth factor (VEGF) in inducing retinal neovascularization has recently

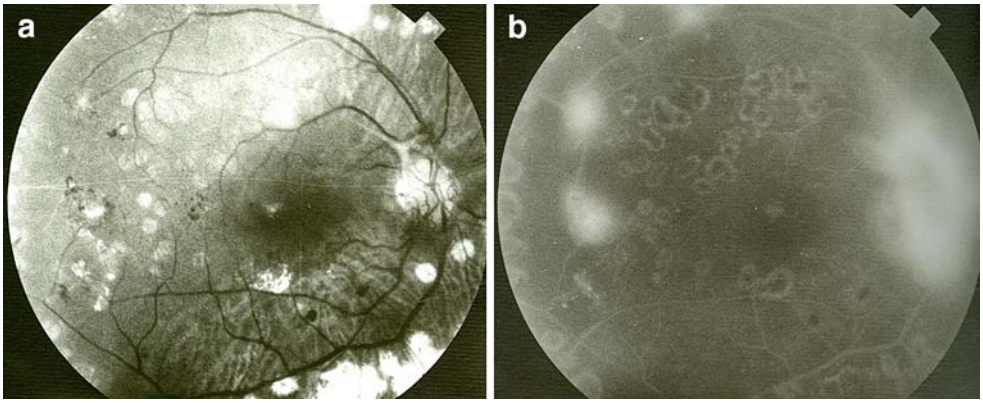

Fig. 1 Mild FPD. **a** Fibrovascular tissue diameter less than 1 disc diameter. **b** Leakage in fluorescein angiography less than 3 disc diameters in late phase

been described [7, 8]. Furthermore, VEGF levels have been found to correlate with the severity of PDR, and successful laser treatment of PDR results in a reduction of VEGF levels [9]. In primates, inhibition of VEGF can prevent iris neovascularization [10, 11]. VEGF-A is a prototype member of the VEGF family, which includes six principal isoforms. Bevacizumab (Avastin, Genentech, San Francisco, CA, USA) is a humanized recombinant antibody that binds to all isoforms of VEGF [7]. Intravitreal bevacizumab (IVB) has recently been reported as a treatment for diabetic iris and retinal neovascularizations [12–16].

In this study, we sought to evaluate the efficacy of IVB in inducing the clearing of vitreous hemorrhage (VH) and the regression of FVT in eyes showing active, progressive PDR.

Materials and methods

The study protocol was approved by the Institutional Review Board of the Ophthalmic Research Center, and all participants gave written informed consent before entering the study.

Eyes with actively vascularized FVT refractory to previous PRP or severe fresh VH precluding completion of PRP were included in this interventional prospective study. Eligible eyes received one to three IVB injection(s) (1.25 mg). In the first group, IVB injection was done at least 3 months after the last session of PRP, and in the second group, IVB injection was performed at least 1 month after the occurrence of VH. Decisions for reinjection(s) were made according to: (1)incomplete resolution of VH, and/or (2) inadequate regression of the active vascular component of FVT to abate the risk of VH. The exclusion criteria were as follows: previous vitrectomy, history of cataract surgery during the past 3 months, history of laser therapy for PDR within the past 3 months, severe lens opacity precluding fundus examination, neovascular glaucoma, intraocular pressure (IOP) more than 21 mm Hg with medication, history of any ocular disease except diabetic retinopathy that might affect visual acuity, advanced diabetic nephropathy (renal disease severe enough to require renal transplantation or chronic dialysis), and finally, uncontrolled systemic hypertension. Complete histories were taken for all patients. The best-corrected visual acuity (BCVA) was measured by Snellen chart recorded in logarithm of minimum angle of resolution(log MAR). Complete ocular examination was performed, including slit-lamp examination for evaluation of iris neovascularization (NVI), IOP measurement, and relative afferent pupillary defect (RAPD). Indirect ophthalmoscopy was performed to estimate the severity of VH and FVT

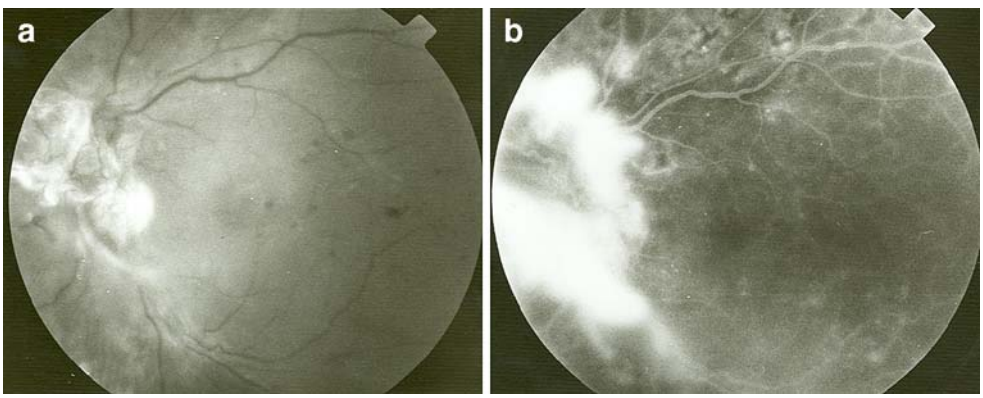

Fig. 2 Moderate FPD. **a** Fibrovascular tissue diameter less than 3 disc diameters. **b** Leakage in fluorescein angiography less than 6 disc diameters in late phase

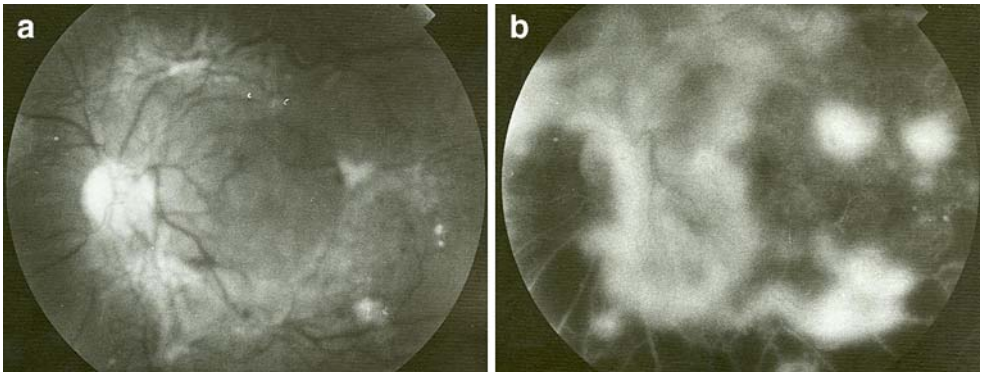

Fig. 3 Severe FPD. **a** Fibrovascular tissue diameter more than 3 disc diameters. **b** Leakage in fluorescein angiography more than 6 disc diameters in late phase

extension. At baseline, fundus photography and FA were performed if the ocular media permitted. Eyes received intravitreal 1.25 mg bevacizumab, and reinjections performed at intervals of 6 or 12 weeks, based on the surgeon's discretion. Follow-up with patients occurred at 1, 6, 12, and 20 weeks after the first injection. Primary outcome measures were the clearing of VH and the regression of FVT. Secondary outcomes were any changes in BCVA and any adverse events.

Severity of VH was graded on the basis of media opacity from 0 to 4+. At grade zero, details of macula could be seen and no VH existed. At grade 1, macular details could not be seen but third-order arterioles were visible. At grade 2, only the second- and first-order arterioles could be seen. At grade 3, only the optic nerve head was visible, and finally at grade 4 only a red reflex from fundus could be seen.

FVT extension was graded as 'mild,' 'moderate or 'severe' on the basis of size in funduscopy or leakage in FA (Figs. 1, 2 and 3).

Statistical method

Numbers and percentages were used to present qualitative data and means ± SD were used to present quantitative data. Paired *t*-test or Wilcoxon test was used to compare data with baseline values. Pearson's or Spearman's correlation tests was utilized for determination of the correlation between quantitative and ordinal variables. All statistical analyses were performed using SPSS 15.0 statistical software.

Results

The study was carried out on 38 eyes of 38 patients (19 male, 19 female) with a mean age of 54.8±10.1 years (range, 21–75 years) with active, progressive PDR. Systemic hypertension was the most common accompanying disorder, and was present in 20 patients. Nine patients had hyperlipidemia, three patients had a history of cataract extraction, and eight patients had a history of renal disease. The mean duration of diabetes mellitus was 18.62± 6.98 years (range, 4–30). All patients had non-insulin-dependent diabetes mellitus.

Twenty-five eyes received one injection, 12 eyes received two injections (nine eyes had the second injection at week six, and three eyes at week 12), and one eye received three injections. No endophthalmitis and no systemic adverse events were observed as a result of the IVB injections.

Anatomical outcome

Severity of VH was found to decrease significantly compared to baseline at follow-up examinations conducted 1, 12, and 20 weeks after the first IVB injection (p=0.014, 0.0001, and 0.002). Borderline improvement was observed at six weeks after the first injection (Tables 1 and 2).

According to univariate analysis, only age had a significant effect on the improvement of VH after 12 weeks: younger patients tended to show a greater degree of resolution of VH.

In comparing the FVT extension relative to baseline, follow-up examinations at 1, 6, 12, and 20 weeks did not

Table 1 Vitreous hemorrhage severity and fibrovascular tissue extension at baseline

VH severity	No (%)
0	6 (15.8)
1	18 (47.4)
2	5 (13.2)
3	5 (13.2)
4	4 (10.5)
FVT extension	No (%)
–*	27 (71.1)
Mild	9 (23.7)
Moderate	2 (5.3)
Severe	0 (0)

*FVT extension could not be evaluated due to media opacity
VH = vitreous hemorrhage, FVT = fibrovascular tissue

Table 2 Visual and anatomical outcomes during follow-up examinations

Time	Baseline	Week 1		Week 6		Week 12		Week 20	
Variable			*P*		*P*		*P*		*P*
BCVA logMAR	1.13±0.84	0.86±0.59	0.033	0.78±0.65	0.035	0.73±0.58	<0.001	0.53±0.35	0.002
VH severity no (%)									
0	6 (15.8)	5 (13.2)	0.014	11 (28.9)	0.06	17 (44.7)	0.0001	28 (73.7)	0.002
1	18 (47.4)	26 (68.4)		18 (47.4)		20 (52.6)		9 (23.7)	
2	5 (13.2)	7 (18.4)		5 (13.2)		1 (2.6)		0 (0)	
3	5 (13.2)	0 (0)		2 (5.3)		0 (0)		0 (0)	
4	4 (10.5)	0 (0)		2 (5.3)		0 (0)		1 (2.6)	
FVT extension no (%)									
–*	27 (71.1)	Not evaluated		24 (63.2)	>0.05	24 (63.2)	>0.05	26 (68.4)	>0.05
Mild	9 (23.7)			12 (31.6)		11 (28.9)		10 (26.3)	
Moderate	2 (5.3)			2 (5.3)		1 (2.6)		2 (5.3)	
Severe	0 (0)			0 (0)		2 (5.3)		0 (0)	

*FVT extension could not be evaluated due to media opacity

show any statistically significant changes (Tables 1 and 2), despite the apparent regression of the vascular component (Figs. 4 and 5).

Two eyes with moderate FVT that received two IVB injections,developed TRD 1.5–2 months after the second injection (Fig. 6). This complication was managed by pars plana vitrectomy that resulted in retinal reattachment.

At the baseline examination, five eyes had NVI, but this decreased to two eyes at 1 week after the first injection. No cases of NVI were detected at week 6, and two cases were seen at week 12; one was a recurrence and the other was a new case. At 20 weeks, however, no case of NVI was visible. Only two eyes with NVI required two injections.

Visual outcome

At the baseline examination, mean BCVA was 1.13±0.84 logMAR (range, 0.1- 2.6). BCVA improved to 0.86±0.59 logMAR (range, 0.1–1.7) at the 1-week follow-up, 0.78± 0.65 logMAR (range, 0.15–2.6) at week 6, 0.73±0.58 logMAR (range, 0.1–1.9) at week 12, and 0.53±0.35 logMAR (range, 0.1–1.2) at week 20 (Tables 2 and 3 and Fig. 7).

These BCVA readings were all statistically significantly improved compared to the baseline (p=0.033, 0.035, 0.001, and 0.002 respectively). In addition, BCVA values at week 6 vs week 1 (p=0.028), and at week 20 vs week 6 (p= 0.021), showed statistically significant increases.

Univariate analysis was performed to detect the factors that influenced final visual outcomes at 20 weeks. Baseline vision (p=0.0001) (Fig. 8), severity of VH at presentation (p=0.0001), and fewer IVB injections (p=0.032) (Fig. 9) had statistically significant effects on the BCVA measured at 20 weeks.

Discussion

This study showed that IVB in eyes with active, progressive PDR can help to significantly clear VH, cause regression the vascular component of FVT, and improve visual acuity. Younger patients tended to show a greater degree of VH

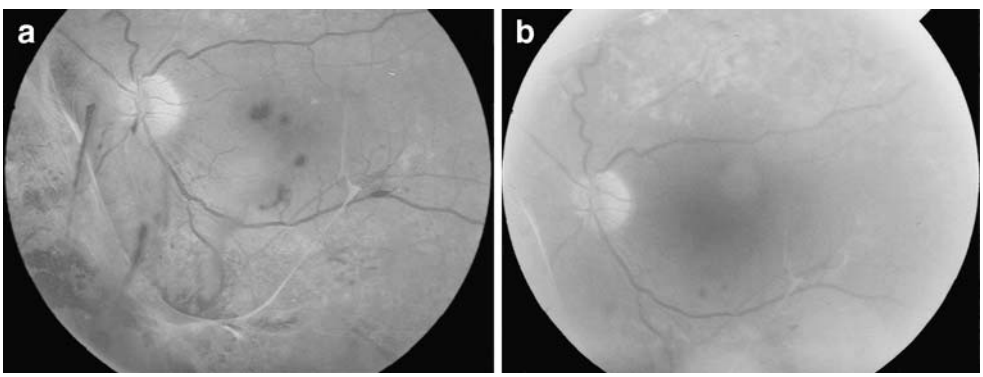

Fig. 4 An eye with active PDR (**a**) before and (**b**) 6 weeks after a second IVB injection

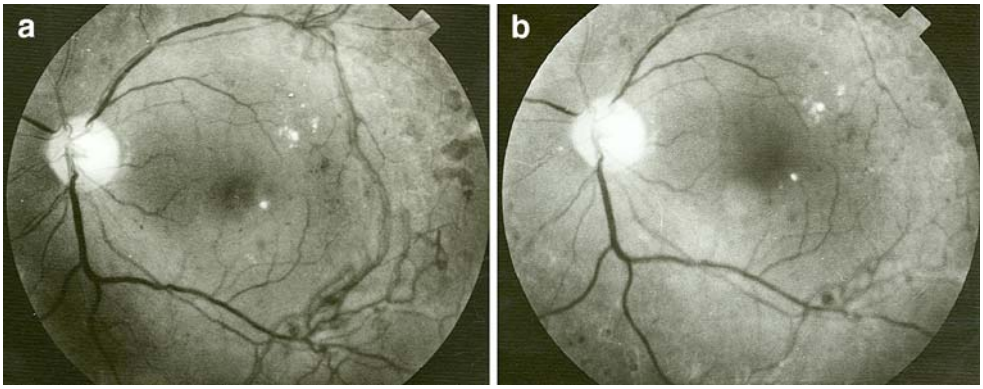

Fig. 5 An eye with active PDR and unremarkable fibrous component (**a**) before and (**b**) 6 weeks after one IVB injection

resolution. The neovascularization in younger patients with PDR is more aggressive, and as a result the response to anti-VFGF therapy may be more prominent. At the same time, IVB in cases with moderate FVT can result in contracture of the fibrous component, leading to TRD. In two of our cases, injection of bevacizumab led to aggravation of retinal traction 1.5 to 2 months later and vitrectomy was required to manage this sight-threatening complication of PDR.

In a recent study, 11 eyes with refractory PDR that had undergone intravitreal injection of 1.25 mg bevacizumab before vitrectomy, developed or showed progression of TRD. The time interval from injection to TRD was a mean of 13 days (range 3–31 days)[17]. Regarding the presence of local TRD in seven eyes of their series before IVB injection, the fibrous tissue was probably more severe than our cases, and this resulted in shorter time interval from injection to TRD relative to our cases. Regarding the paucity of this complication, we could not differentiate the natural course of PDR from the effect of IVB in formation of TRD in these eyes.

Of various pharmacologic agents affecting the development and progress of diabetic retinopathy, those blocking VEGF may play a significant role in stabilizing and preventing retinopathy progression [4]. The biologic effects of IVB in patients with retinal and iris neovascularization secondary to diabetes mellitus were evaluated [12]. Forty-five eyes from 32 patients with retinal or iris neovascularization secondary to diabetes mellitus received IVB (6.2 μg-1.25 mg). FA showed that all patients with neovascularization had partial or complete reduction in leakage of the neovascularization within 1 week after the injection. Complete resolution of angiographic leakage of neovascularization of the disc was noted in 19 of 26 eyes (73%), and leakage of iris neovascularization completely regressed in nine of 11 eyes (82%). The leakage was seen to diminish as early as 24 hours after injection.

Our study showed that IVB can accelerate the resolution of VH and thereby lead to visual improvement. Spontaneous resolution of VH in diabetic patients usually takes time, mainly because of continuous bleeding from active neovessels [13]. Vitreous surgery can be performed in these eyes, to remove the blood and FVT and perform laser photocoagulation in the same session [2, 3]. However, not all diabetic patients can tolerate the burden of this major procedure, due to their poor systemic condition. Following

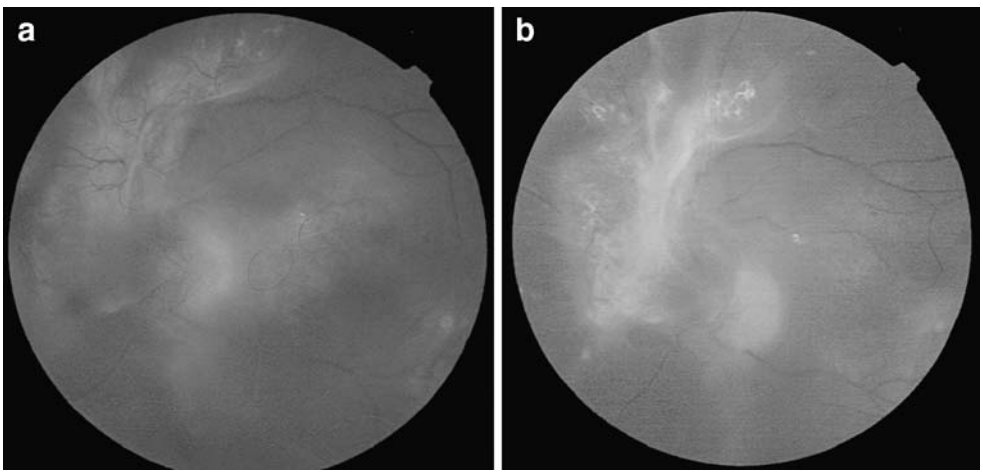

Fig. 6 An eye with active PDR with moderate fibrous component (**a**) before and (**b**) 6 weeks after the second injection, which led to aggravation of TRD and necessitated pars plana vitrectomy

Table 3 Changes of Snellen visual acuity during follow-up examinations

P-value	20/40≤ no (%)	20/200–20/40 no (%)	20/400–20/200 no (%)	< 20/400 No (%)	Time
__	5 (13.2)	16 (42)	5(13.2)	12 (31.6)	Baseline
0.033	10 (26.4)	16 (42)	-	12(31.6)	Week 1
0.035	14(36.8)	14(36.8)	1(2.6)	9(23.8)	Week 6
0.001	16(42)	14(36.8)	1(2.6)	7(18.6)	Week 12
0.002	17(44.75)	17(44.75)	4(10.5)	-	Week 20

IVB, regression of the neovascularization and subsequent cessation of bleeding occurs while resorption of hemorrhage continues. This results in clearing of VH and improvement of vision. In addition, achievement of media clarity helps the retinal specialist complete the scatter photocoagulation, or perform additional laser therapy to ensure that the neovessel regression lasts.

In a study [13], two patients with VH due to PDR were treated with at least one intravitreal injection of 1.25 mg bevacizumab. Both patients experienced improvement in visual acuity starting within the first week after IVB injection. The VH in each patient showed partial resolution at 1 week and nearly complete regression at 1 month. Similarly, in our patients, the severity of VH decreased significantly at week 1 after the first injection, and visual acuity improved as well. The severity of VH became slightly aggravated at week 6 in our series. This correlated with the disappearance of the anti-angiogenesis effect of IVB and prompted repeat injections of IVB.

Persistence of active neovascularization is the cause of sight-threatening complications of diabetic retinopathy; these complications include VH, fibrovascular proliferation, and TRD [14].

In a study [15], the short-term effects of a single 1.5 mg IVB for management of persistent new vessels refractory to laser treatment were evaluated using FA and visual acuity tests. Neovessel extension was calculated objectively from leakage area. The authors concluded that IVB achieves short-term reduction of fluorescein leakage from persistent active neovascularization in patients with diabetic retinopathy. This study is the most similar to ours in its inclusion criteria and outcome measures, though none of the patients in that work had VH. In both studies, BCVA improved 1 week after the first injection, and persisted through the last follow-up examination at week 20.

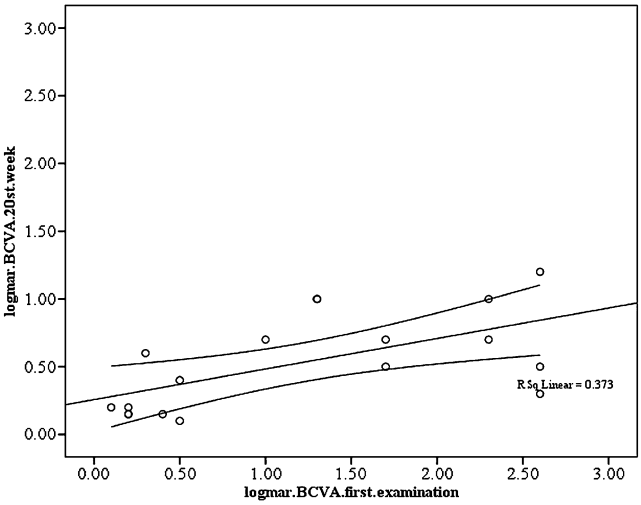

Fig. 8 Correlation of better baseline vision with better final vision at week 20 follow-up

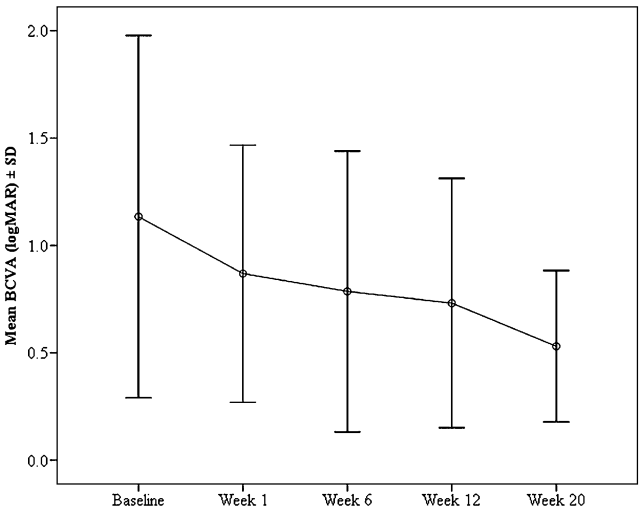

Fig. 7 Changes of best-corrected visual acuity during follow-up examinations

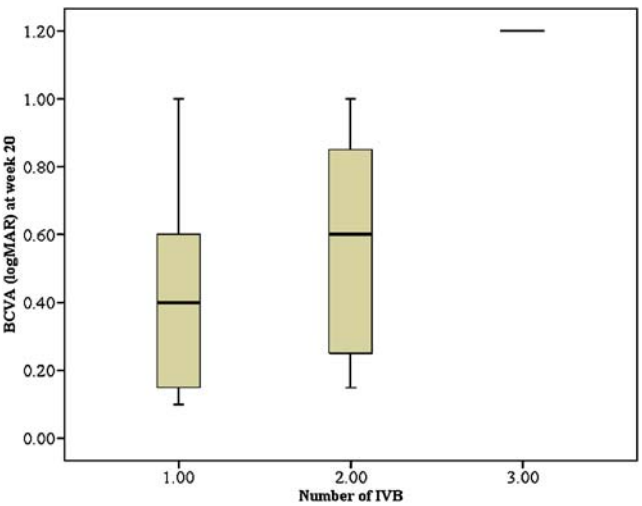

Fig. 9 Correlation of better visual outcome with fewer injections

In a case report [16], rapid regression of iris and retinal neovascularization after IVB was reported in a diabetic patient.

In another study [18], a case was presented for rapid improvement of NVI from a single IVB injection. Our series included five eyes with NVI, two of which persisted up to 1 week after the first injection, but completely regressed at week 6. At week 12, one of our previous cases and a newly diagnosed one were detected, but both again completely regressed by 20 weeks.

In summary, IVB has a significant effect on clearing VH, and as an adjunctive treatment may allow the completion of PRP in eyes with active, progressive PDR. This procedure may be especially relevant to diabetic patients, who are high-risk candidates for extensive surgical intervention. Since IVB may exacerbate the contraction of the fibrous component of FVT, it should be injected prior to the significant growth of fibrous tissue or a planned vitrctomy should be considered a few days after injection.

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